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A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1934

By
ERNEST HOCKING RUNYON

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RATIO OF WATER CONTENT TO DRY WEIGHT IN LEAVES OF THE CREOSOTE BUSH

ERNEST H. RUNYON

(WITH NINE FIGURES)

Introduction

The general characteristics of the remarkably drought resistant creosote bush, *Larrea tridentata* Cav., have recently been discussed and the structure and life history of the leaves portrayed (**23**). Part of the foliage is persistent through long drought. The same leaf may actually have two periods of growth separated by a season of drought. In the work here reported it was found that this drought resistant foliage is characterized by an exceptionally low water content and high saturation deficit. Other foliage on the same branches is unable to withstand a decrease in moisture content much below a level characteristic of many mesophytic woody plants. By a simple method it is shown that leaves with the same moisture content may differ markedly in their access to a water supply. The investigations were made in the vicinity of the Desert Laboratory of the Carnegie Institution of Washington, Tucson, Arizona, during the summers of 1928–31.

Literature

Apparently no special study of the water content of *Larrea* has previously been made. LIVINGSTON and BROWN (**15**), the first to publish data in this country concerning water content changes in leaves, give four measurements for *Larrea* foliage (showing a change not confirmed by the present work). SCHRATZ (**25**), in a study of various aspects of the water physiology of Arizona desert plants, includes 12 moisture determinations on leaves of *Larrea* for the purpose of indicating the influence of time of day and of habitat. ASHBY (**1**) has added three more determinations to this short list. The data presented in these papers indicate that the foliage has a low and fluctuating water content, which may be from about one-half to two times the dry weight.

Prominent in the general literature pertaining to the water balance of plant tissues are the publications of YAPP and MASON (41), WALTER (40), MAXIMOV (18), PISEK and CARTELLIERI (22), and STOCKER (31–35). Earlier literature is reviewed by these authors. While many of the methods employed in these studies are open to certain criticisms, the facts in regard to water content stand out prominently. Each species has a somewhat characteristic water content which changes within limits which are also rather well defined. In some species, especially those of dry habitat, changes are very marked; in others, especially shade mesophytes, the water content remains well poised. In any habitat, however, there are likely to be both types of plants, some physiologically capable and some incapable of withstanding wide variations of water content. Inasmuch as fluctuations are due to a varying balance between water intake and loss, they form a valuable index to the water conditions of the plant.

True water content change, however, is rarely measured. Change of *percentage* water content is another quantity. Most often, percentage water is interpreted simply as absolute water content. This may result in magnification or in masking of the true changes of water content. Thus, for example, differences in actual water content are probably less than differences in some of the percentage data of KRASNOSSELSKY-MAXIMOV (19) and of SCHRATZ (25), because of changes of dry weight in direction opposite to those of water. YAPP and MASON (41) even draw conclusions as to turgor differences from their percentage data. The experimental conditions in some of YAPP and MASON's experiments were certainly such as to induce marked dry weight changes, exaggerating water content differences. MAXIMOV and KRASNOSSELSKY-MAXIMOV (19) have shown in one case how the rapid disappearance of organic reserves in wilting leaves may prevent their showing a percentage water content below that of turgid controls.

Several investigators, using plants of high moisture content (5 to 10 times the dry weight), have expressed their data as percentage of the *fresh weight.* When this is done, extensive changes in actual water content make comparatively small changes in percentage water content, as may be seen in figure 1. The water percentage of the fresh

weight changes progressively less and less as the absolute water content increases if the dry weight is constant. Water as percentage of the dry weight, on the other hand, is a linear relation of the absolute water content (fig. 1). The masking effect of the fresh weight basis of computation makes it seem possible that the actual water content changes in the plants investigated by KNIGHT (12) and by GILBERT and ADAMS (8) may have been greater than is indicated by their data.

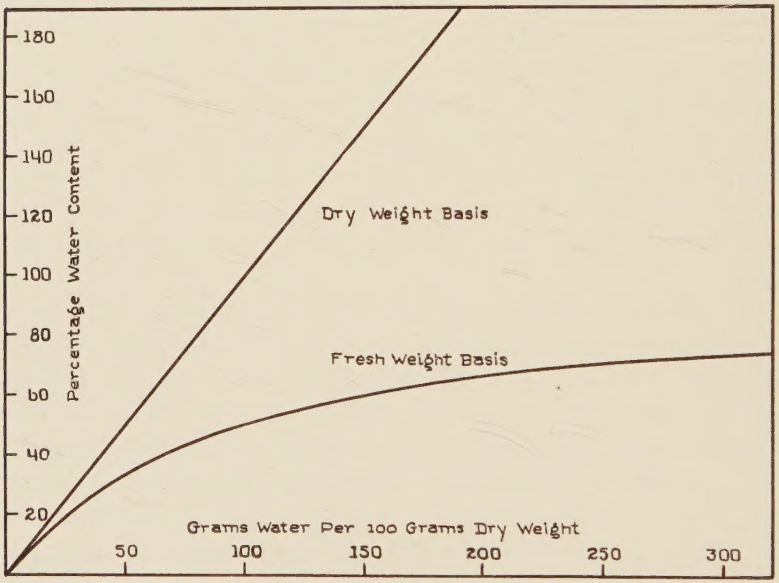

FIG. 1.—Relation between water content and percentage water content. Comparison of dry and fresh weight bases of computation.

The moisture content does change even in shade plants. Percentage water content data are supported by data obtained by other methods, as by volume changes (2, 36) and the difference between absorption and transpiration (29). DENNY (7) has followed diurnal changes in leaves by methods which permit approximate determination of absolute changes. He has demonstrated fluctuations in absolute water content so great as to show that the fresh weight basis of comparison is unsuitable for most of the varieties (about 20) he used. The variations in absolute water content might have been greater had he collected his material before 4 P.M., when the mini-

mum percentage water content frequently occurs. In the woody plants DENNY used, the dry weight did not change appreciably during the night. The numerous correlations which have definitely been established between percentage water content changes and changes in the water conditions are further evidence that true water content changes are responsible for much of the observed percentage changes.

On the other hand, water content is affected not alone by water conditions. Osmotic, hydrational, and wall pressures, which influence the water content of a cell, may be markedly affected by the nature and concentration of the ions present. Water content will therefore vary with the nutrient conditions of the habitat (14). Differences in the water conductivity of stems may be expected to influence the water content of leaf cells (28). Flowering or tuber formation may possibly influence the amount of foliar moisture (4, 3, 10). Finally, light undoubtedly influences the capacity of cells to hold water. Interpretation of the meaning of water content data must therefore be made cautiously. Nevertheless, especially in desert regions where all vegetation is dependent on its water conditions, nutrient and other differences (although of unquestionable influence) are probably of minor importance as compared with water supply in affecting water content.

A small number of water content measurements on any one species can have little significance because of the likelihood of sampling errors and because meaning of the data procured is obscure without bases of comparison, that is, standard water contents associated with some definite conditions or activities of the plant. For interpretation of the existing water content, several investigators have compared it with the value attained when leaf cells are brought to approximate saturation with water (37, 11, 26, 4, 31–35, 21, 22). For attainment of saturation, shoots are usually put in moist chambers, their cut stems in water, for 12 to 72 or more hours. VASSILJEV (39) objects to this method in that it leads to values which are too high; periods as long as three days in a moist chamber probably do lead to erroneous results. However, the accumulated data give some idea of the degree to which the cell walls normally are distended. Some plants are always far below saturation; others suffer injury if their water content is reduced much below their maximum water holding

capacity. The difference between the percentage water content at saturation and the existing percentage water content has usually been spoken of as the saturation, or water deficit.

OPPENHEIMER (21) recommends determination of sublethal water content, which is the percentage of the saturation water content which may be lost before death of detached leaves or shoots left on a laboratory table perhaps two or three weeks. The likelihood of distinctly pathological effects would seem to restrict the utility of this method. Where measurable, a much more reliable and significant water content is that at permanent wilting (5, 13, 17). Even this minimum water content may be rather indefinite (20), and is not determinable for a plant like *Larrea*, which never wilts and normally shows large changes in moisture content.

Knowledge of the degree of hydration of the chloroplasts, cytoplasm, nucleus, or other portions of the protoplast would be more significant than knowledge of the total "free" water content of tissues. However, as yet we have no method for determining the water content of even the protoplasm alone as distinct from the associated non-living portions. The finest methods are gross in comparison with the intricacy of organization of the living plant.

Methods

Leaves are picked from the bush and put into tared weighing bottles or vials, which are then kept tightly stoppered and as cool as possible. The extreme importance of careful sampling will be evident from the data presented. The inclusion of parts other than leaves (twigs, galls, flowers, or fruits) may introduce considerable error. If weighing cannot be made immediately after picking the leaves, the bottles are inclosed in a humid box. Weighings are made to milligrams. More accurate weighing is unnecessary (except for very small samples) in view of the magnitude of variations inevitable in sampling. After weighing the leaves, the stoppers are removed and the leaves heated in an electric oven for about 24 hours. In most cases leaf samples are completely dry in less than 12 hours, but as one day is a more convenient interval, and since no appreciable change in weight of *Larrea* leaves occurs even with much longer heating, the 1 day period has been adopted. At no time in Tucson

has it been found necessary to use a desiccator; the dry leaf material is not sufficiently hygroscopic to increase significantly in weight even after standing open to the air for 20 minutes. Weighings, however, are always made immediately after the stoppered containers have sufficiently cooled.

To emphasize the possibility of a varying dry weight, data are given for the most part as the ratio of water content to dry weight (wc:dw) instead of in percentage figures. Percentage water content is of course 100 times the value of the ratio. Wc:dw ratios at different times of day, at different seasons, at water saturation, and when transpiration is eliminated are compared. The special methods required for the determination of these ratios are described in their respective sections.

I. The wc:dw ratio under natural conditions

Leaves of different age.—The creosote bush has an abundance of fine branches. One side branch is usually to be found at each node. Under favorable conditions, that is, ample soil water, every branch is densely clothed with tiny leaves, two at a node for seven to ten nodes from the apex. The wc:dw ratio of leaves of different age as found contemporaneously at these different nodes is markedly different. The sampling method used to determine this was to select several (6–20) leafy shoots, and to put all the buds in one weighing bottle and all of the leaves at each successively lower node into separate weighing bottles. In some cases all of the leaves were divided into only two or three groups, each group containing the leaves of two or more nodes from each of the several twigs. All of the data obtained fall into either one or the other of two contrary groups, whose characteristics are summarized in tabulation shown on page 524. Figure 2 shows graphically data selected from the two groups. These two seemingly irreconcilable situations will be discussed later.

Clearly, since so great differences in percentage water content are to be found even at adjacent nodes of *Larrea* stems, uniform successive samples from the same bush can be obtained only by careful selection of leaves of exactly the same age at each picking.

Leaves from different branches of same bush.—The leaf wc:dw ratio of one branch may or may not be closely similar to that

of other branches. Differences as great as 20 per cent may exist between different branches on the same bush. Even when branches are selected for close uniformity, as when two forks ("twins") of a Y-shaped branch are separately sampled, differences as great as 5 per cent are sometimes shown. By very careful selection closer replication is usually obtainable, but in following progressive changes of the wc:dw ratio, differences less than 5 per cent are of doubtful significance.

DATA OF GROUP I	DATA OF GROUP II
—show the wc:dw ratio to be higher at each successively lower node; that is, the ratio increases with age.	—show the wc:dw ratio to be lower at each successively lower node; that is, the ratio decreases with age.
—show a rather consistent uniformly ascending gradient from one node to the next below.	—show not so consistently a uniformly descending gradient from one node to the next below.
—comprise all the determinations made on dates other than Aug. 9–12, 1931, namely, 9 dates between June 15 and Sept. 2, including periods of drought dormancy as well as periods of rapid growth.	—comprise all the determinations made on the four consecutive days Aug. 9–12, 1931, and none other,[1] a period of very rapid growth.
—show wc:dw ratios of 0.63–1.68.	—show wc:dw ratios of 1.20–1.98, the latter being the highest recorded for *Larrea* in the present investigations.
—comprise 63 determinations, involved in 18 tests, made on 12 bushes.	—comprise 60 determinations, involved in 14 tests, made on 7 bushes.

[1] In two of the three tests at the University of Cincinnati, greenhouse-grown *Larrea* showed the maximum wc:dw ratio in the buds. The water content of the buds and youngest leaves was more than twice the dry weight.

VARIATIONS WITH SEASON AND HABITAT.—The purpose of these investigations was to determine the nature of the existing variations of the wc:dw ratio. Except in a general way, no attempt was made to correlate these variations with environmental factors.

Adjacent bushes in an apparently uniform habitat frequently deviate conspicuously in water content, indicating probable local differences in water conditions. Thus for example, within an area

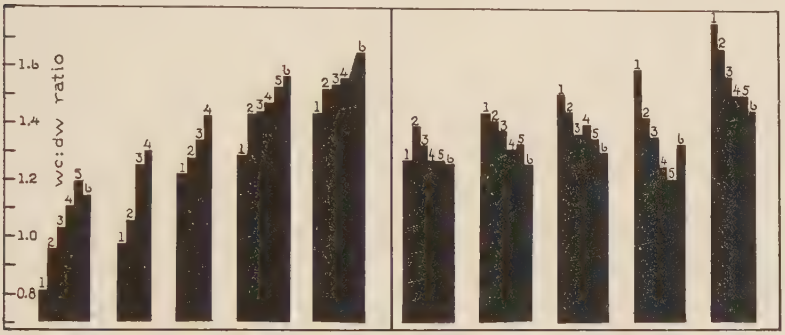

FIG. 2.—The wc:dw ratio of leaves at different nodes. Each diagram represents the relative wc:dw ratio of leaves at successive nodes (1, 2, 3, 4, 5, 6) from apex. The five diagrams at the left represent the usual situation, the oldest leaves having the highest wc:dw ratio; the five diagrams at the right show the reverse situation, which occurred during a period of rapid growth.

of about an acre on Tumamoc Hill six individuals were found to have wc:dw ratios of from 0.71 to 1.13; in another locality six bushes ranged in wc:dw from 0.77 to 0.97. In these tests leaf samples were taken from all parts of the selected bushes, as nearly simultaneously as possible, sometimes with the aid of an assistant. The differences shown are for the most part unpredictable from external appearances, although brighter green foliage frequently has higher percentage water content.

The wc:dw ratios for leaves of bushes in habitats which are obviously different are correspondingly different. In July, 1931, on Tumamoc Hill there had been a number of rains and the ratio was greater than 1.0 (1.10–1.33). At the same time, 8 miles to the south (Ajo Pass, Tucson Mountains) where no considerable rain had fallen, the ratio was little more than half this amount (0.54–0.67). Similar variations occur in the same bush before and after the summer rains.

Determinations made in late June and early July, 1928, on bushes in the vicinity of the Desert Laboratory, showed their average ratio to be 0.54. Following the midsummer rains, the average was 1.35. These data represent 77 tests on 18 bushes. In table I are other specimen ratios of individual bushes before and after periods of rain or watering. Between one growing season and the next, weeks or even months of excessively dry hot weather may intervene, while the percentage water content of all leaves retained on the twigs drops

TABLE I

SEASONAL VARIATION OF WC:DW RATIO

Bush	Before rain period		Precipitation (cm.)	After rain period		Percentage increase
	Date	wc:dw		wc:dw	Date	
L.....	7/26/28	0.59	8.7	1.15	8/15/28	49
8.....	7/31/28	0.59	4.9	1.12	8/ 9/28	47
Bj.....	7/15/31	0.60	10.4	1.30	8/27/31	54
P.....	7/26/28	0.71	6.6	1.30	8/ 9/28	45
b*.....	7/31/30	0.74	*	1.25	9/26/30	41
y*.....	7/27/30	0.89	*	1.95	8/14/30	54
a*.....	7/ 4/30	1.04	*	1.56	7/31/30	33

* Artificially watered.

sharply. If the dry period is long and severe, the water content may fall to less than one-half the dry weight.

From the data on nodal and seasonal differences, we may conclude that a leaf ordinarily undergoes an increase in percentage water content as it comes to maturity, and then a decrease due to seasonal drought.

DIURNAL VARIATIONS.—Several hundred ratio measurements were made during the summers of 1928, 1930, and 1931 in order to obtain a complete picture of the changes that take place from hour to hour of the day and night. Since the simultaneously existing differences from node to node or from branch to branch are often greater than in the same leaves from hour to hour, sampling must be done with the greatest care. One satisfactory procedure is to select several shoots which by previous test have proved to have nearly identical leaf wc:dw ratios, and which have the same exposure, density of

foliage, etc.; to use then the leaves of one shoot for each time of day a sample is desired.

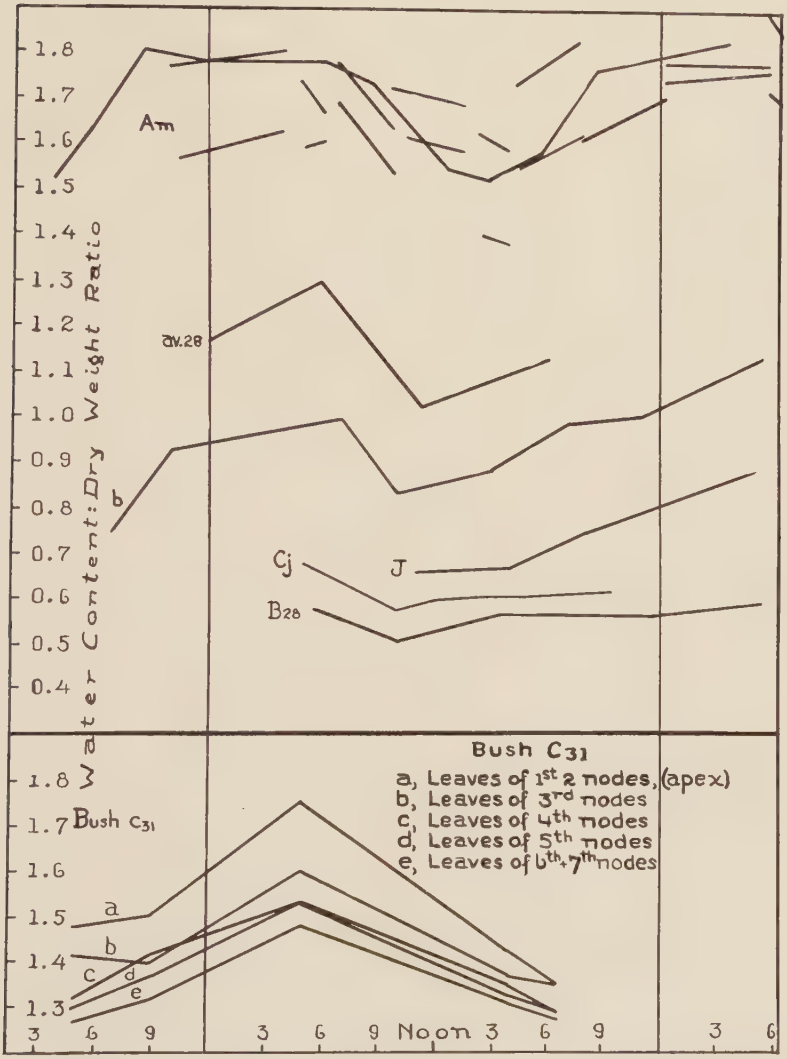

FIG. 3.—Diurnal variation of wc:dw ratio of leaves of different bushes

Graphs representative of the most carefully executed experiments are shown in figure 3. Many other graphs obtained follow the same

curves; a few are of different form, or show erratic fluctuations. The latter results are probably due partly to faulty sampling, and partly to obscure circumstances which derange the normal changes. The curves for bush Am in figure 3 consist of (1) a continuous line curve each point of which represents the water-dry weight ratio of the leaves of a single twig, all of the twigs having been chosen for strict uniformity; and (2) short lines, each of which joins the wc:dw ratio of half of the leaves of a selected shoot at one time and the wc:dw ratio of the remaining leaves 2–6 hours later. The short lines rarely coincide in position with that of the continuous line curve, indicating again that the level of percentage water content may be markedly different in various parts of a bush. It is significant that the same general direction of change appears consistently in all the curves for bush Am. The changing ratio in leaves *at different nodes* is shown in the curves for bush C31. Here all the shoots used had been shown to be uniform in wc:dw ratio. It will be seen that these readings were taken when all bushes tested had a lower ratio in the lower leaves. The other curves of figure 3 represent the average condition of leaves of all ages, and taken from all parts of the bushes indicated. It is clear that inasmuch as these do not show the variations at different nodes and branches, they represent an incomplete picture. A composite of curves must be given to express diurnal variation adequately.

The wc:dw ratio is greatest at daybreak. A sharp decline in the ratio begins at sunrise, leading to a minimum value which may occur at any time between 10 A.M. and 7 P.M. If the minimum occurs early in the day, there is a gradual increase during the afternoon or a low fluctuating ratio until sunset when there starts a steady increase. The difference between the maximum and minimum values for any 24 hour period is from about 0.03 to 0.60, or 10 to 35 per cent of the maximum value.

SEPARATE RÔLES OF WATER CONTENT AND OF DRY WEIGHT IN CHANGES OF THE WC:DW RATIO.—It is to be expected that dry weight changes, if any, are in a direction opposite to those of water content. This follows from a consideration of the usual periodicity of transpiration and photosynthesis. Some evidence in regard to the occurrence of dry weight changes in *Larrea* is afforded by I_2KI-starch

tests made on leaves picked at different times of day. It is found that leaves of irrigated bushes contain conspicuously more starch at 5 P.M. than at 5 A.M. Leaves of bushes not irrigated contain very little starch, however, and exhibit a very inconspicuous difference in the starch content at 5 P.M. and 5 A.M. A change in starch content does not necessarily mean a change in dry weight. But there is a suggestion here that leaf dry weight changes may be more pronounced in bushes amply supplied with water.

A number of attempts were made in the field to determine how much change takes place in the absolute dry weight and water content from hour to hour. In order to do this, one leaf of the pair at each of several nodes was picked at one time, and the other leaf at the same nodes, closely matched as to size and exposure, picked at the second time. Obviously success in such work, especially with such small leaves, requires the utmost care to insure exact replication. Uncontrollable environmental conditions and limited time prevented satisfactory results in Tucson, in lieu of which similar experiments were undertaken at the University of Cincinnati under more favorable, but artificial, conditions, and with very limited material. The plants used were in pots placed out of doors and fully exposed to the sun and wind. The results are given in tables II and III.

As seen in the tables, the two leaves at the same node are designated *a* and *b*. In further experiments, the half-leaf method was used, the two pinnae of the same leaf being separately tested and designated *a* and *b* respectively. Table II shows how closely similar *a* and *b* are when sampled simultaneously. Table III gives the values at about the time the ratio is at maximum and at minimum. Although the data in the tables are meager, some facts seem clear:

1. Except in experiment 1 (in which only three leaves were included in a sample) and in experiment 3, the two leaves at the same node or the two halves of the same leaf as sampled simultaneously differ from each other less than 3 per cent.

2. In experiment 3, the higher difference (4.4 per cent) in the dry weight and milligrams of water does not appear in the percentage water content, making it seem very probable that equivalent areas were not included in *a* and *b;* that is, that there was an error in sampling.

3. The differences shown in table III are greater than those in table II. They are, moreover, in the expected directions. The data are therefore sufficient to demonstrate a diurnal change in dry weight, as well as in water under the conditions of the experiments.

4. Except in experiment 6, the change in water content is greater than the change in dry weight.

TABLE II

COMPARISON OF THE TWO LEAVES (A AND B) AT SAME NODE AND OF THE TWO HALVES (A AND B) OF SAME LEAF (UNIVERSITY OF CINCINNATI)

Experiment no. and method	Dry weight			Water content			Water content (% dry weight)		
	A (mg.)	B (mg.)	Percentage difference*	A (mg.)	B (mg.)	Percentage difference*	A (%)	B (%)	Percentage difference*
Opposite leaves (a and b):									
1. Leaves of 3 nodes	5.8	5.6	3.50	12.1	12.7	4.84	209	227	8.25
2. Leaves of 6 nodes	8.8	8.9	1.13	19.1	19.0	0.52	217	214	1.39
Average per leaf (9 nodes)	1.622	1.611	0.68	3.467	3.522	1.56	214	218	1.85
Half leaves (a and b):									
3. 10 half leaves	11.5	11.0	4.44	20.8	19.9	4.41	181	181	0
4. 10 half leaves	13.1	13.2	0.76	26.3	26.8	1.88	201	203	0.99
5. 15 half leaves	17.3	16.8	2.93	32.1	31.7	1.88	187	189	1.06

* In percentage of $\frac{a+b}{2}$.

5. Water content and dry weight changes are in opposite directions as predicted. The wc:dw ratio therefore changes more than does the actual water content.

Further information regarding the diurnal changes in *Larrea* leaves is afforded by study of the osmotic concentration of the cell sap.

DIURNAL CHANGE OF OSMOTIC CONCENTRATION AND WC:DW RATIO.—Dr. T. D. MALLERY and the writer conducted an experiment to determine the nature of the diurnal fluctuation of osmotic and wc:dw ratio values (**16**). Two experimental bushes, Am and Bm, were selected as representing contrasting foliage characteristics.

Recent rains had provided enough soil water to permit growth, but Bm appeared less favorably situated than Am. The leaf size and rate of growth were evidently greater for bush Am. (WALTER's (40) figure 26, p. 65, shows Am). At intervals of 2–4 hours for 50 hours, leaf-plus-twig samples were taken from all over the bushes for osmotic and water content data. Simultaneously, leaf samples from care-

TABLE III

DIURNAL CHANGE OF DRY WEIGHT, WATER CONTENT, AND PERCENTAGE WATER CONTENT (UNIVERSITY OF CINCINNATI)

EXPERIMENT NO., METHOD, AND TIME INTERVAL	DRY WEIGHT (MG.)			WATER CONTENT (MG.)			WATER (% OF DRY WEIGHT)			WATER (% OF FRESH WEIGHT)		
	A	B	CHANGE*	A	B	CHANGE*	A	B	CHANGE*	A	B	CHANGE*
Opposite leaf method:												
6. 9 pairs of leaves 5:00 P.M.(=a) 4:30 A.M. (=b)	23.6	21.7	−8.4	37.9	39.1	+ 3.1	160.0	180.0	+11.8	59.5	64.8	+8.5
Half leaf method:												
7. 10 leaves 5:00 P.M.(=a) 4:30 A.M. (=b)	9.5	9.0	−5.4	14.0	16.6	+16.3	147.0	184.0	+22.2	61.6	64.3	+4.3
8. 10 leaves 5:00 P.M.(=a) 4:30 P.M. (=b)	11.9	11.4	−4.3	18.8	21.3	+12.2	158.	187.0	+16.4	61.2	65.1	+6.2
Average (6+7+8) 5 P.M.–4:30 A.M.	15.0	14.0	−6.5	23.6	25.7	+ 8.2	155.0	184.0	+17.0	60.8	64.7	+6 2
9. 13 leaves 6:00 A.M.(=a) 3:30 P.M. (=b)	16.3	17.0	+4.2	31.6	27.4	−14.2	194.0	161.0	−19.0	66.0	61.7	−6.7

* In percentage of $\frac{a+b}{2}$.

fully selected individual branches were taken for wc:dw ratio determination. Some of the results are plotted in figure 4 (*cf.* MALLERY's fig. 5).

Evidence in regard to diurnal change of dry weight lies in the constancy of the product: osmotic value times wc:dw ratio (22). The product may be designated thus:

$$\frac{kc}{Ws}\left(\frac{Ws+Wr}{c+d}\right),$$

where c = number of millimols of osmotic species, (c+d) = grams dry weight, Ws = grams of solvent water, (Ws+Wr) = grams total water content, and k = a constant. If the solvent water and the total water content are almost equal in the leaf tissue, that is, if Ws and (Ws+Wr) in the product balance each other, then it follows that the product is not much affected by changing water content. On the other hand, total dry weight is undoubtedly considerably greater

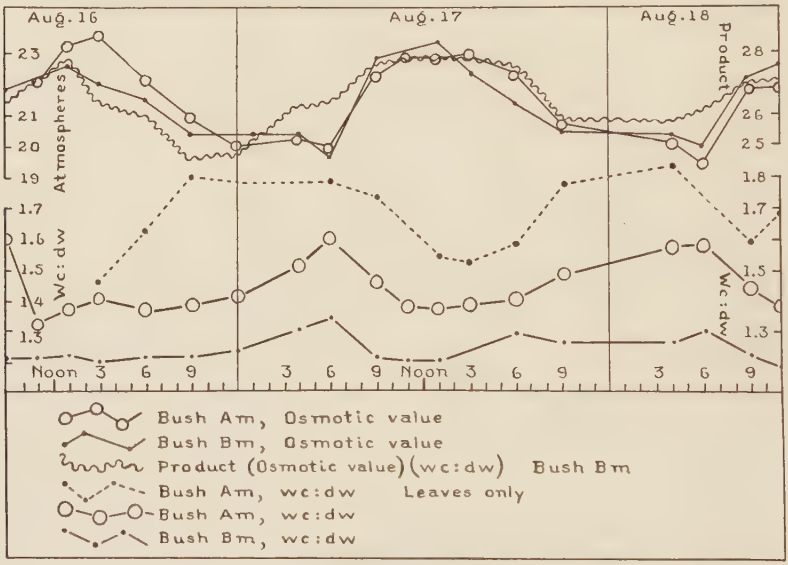

FIG. 4.—Diurnal changes in foliage (leaves plus green twigs) of two bushes, Am and Bm.

than the osmotic portion of it, so that if the product varies considerably, the dry weight is changing (as well as, probably, the water content).

In the present experiment the product varied quite as much as did the factors of the product (fig. 4). According to the foregoing, this means that in these bushes diurnal changes of the dry weight were occurring. Since the validity of the assumptions involved in this interpretation is open to some question, the data must be considered not as proof but merely as evidence additional to that presented in the previous section, pointing to the same conclusion, that diurnal dry weight fluctuations do occur in *Larrea* bushes well supplied with water.

The course of the diurnal changes need not be discussed further than (1) to call attention to the inverse direction of change of osmotic and ratio values, (2) to note that the interpretation (as by WALTER 40) of such a relationship to mean that water content changes are the cause of the osmotic changes is unjustified in view of the probable dry weight fluctuations, and (3) to note that the changes shown are in the directions which would be expected in view of the periodicity of photosynthesis and transpiration.

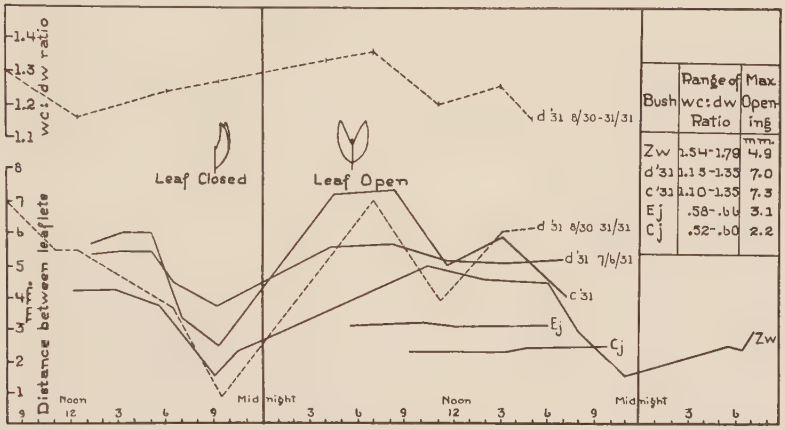

FIG. 5.—Diurnal movement of *Larrea* leaflets and representative curve of the march of percentage water content. No relation exists between time of leaf opening or closing and level of percentage water content.

LEAFLET MOVEMENT; RELATION TO DIURNAL CHANGE IN WC:DW RATIO.—*Larrea* exhibits nyctinastic leaflet movements. In the hope of finding a correlation between the march of diurnal water content variations and the movements, as reported by TRELEASE (38) for palm leaves, exact measurements of the positions of *Larrea* leaflets were made at various times of day and night, and concurrently, wc:dw ratio determinations on similar leaflets. No correspondence appeared, however. The time of maximum opening (7–11 A.M.) occurs two or more hours after the time the water content is at its maximum (sunrise); and the time of minimum opening (7–11 P.M.) occurs many hours after the time of day water content is at a minimum (fig. 5). Evidently the changes in water content responsible for the movements are localized in the motor tissue, and are not general throughout the leaf.

General discussion.—*Larrea* foliage exhibits some striking characteristics. Most outstanding are the changes that may take place in the life cycle of a single leaf, and the lowness of the water content. Analysis of the wc:dw data here reported clarifies and amplifies the concepts previously presented (**23**) showing *Larrea* to have a peculiar admixture of xeric and mesic characteristics.

During the growing season the leaves have a water content level almost identical with that of leaves of such genera as *Quercus*, *Castanea*, *Hamamelis*, and *Fagus* (**27, 6**): between one and two times the dry weight. Indeed most of the foliage at this season in water content as well as in structure gives no evidence of the xeric nature of *Larrea*. Drought resistance is characteristic of only a small proportion of the leaves.

It is possible to construct diagrams showing roughly the wc:dw ratios for representative pairs of leaves that develop successively on a twig, and the change in the ratios from one season to the next during the summer (fig. 6). This is done by correlation of the nodal and seasonal wc:dw ratio data with the life duration and growth studies (**23**), in which it was established that seasonal changes represent not merely a substitution of new leaves for old ones, but an actual change in the same leaves from one season to the next. For convenience, and according to their time of development, three types of leaves, *a*, *b*, and *c*, may be distinguished.

The *a* type of leaf begins its development early in a growing season and is thus subject during its whole developmental period to relatively mesic conditions. Loss of much water is fatal to leaves of the *a* type, and they therefore fall early in the dry period or early in the next growing season. The data on nodal variation show that younger leaves regularly have a lower water content than older ones. The leaves designated *b* and *c* are therefore represented in figure 6 with narrower bands than the *a* leaves, which have previously expanded from the bud. Examples of the *b* type of leaves occur infrequently. They do not attain full size before the dry season checks further growth. They survive the drought and show increased water content but no further growth during the second growing season. It is the *c* leaves which have two periods of growth. These leaves begin their development during a period of increasing drought, last through the dry season with the lowest water content of any of the

foliage, and on the return of rain, not only increase their water content but resume growth. They are shed in the subsequent dry period. The three leaf types listed in the order *a*, *b*, and *c* are arranged according to increasing life duration, increasing drought resistance, and decreasing water content. Of all the leaves, the *a*1 subtype (fig. 6) is most abundant. They have a comparatively high wc:dw ratio

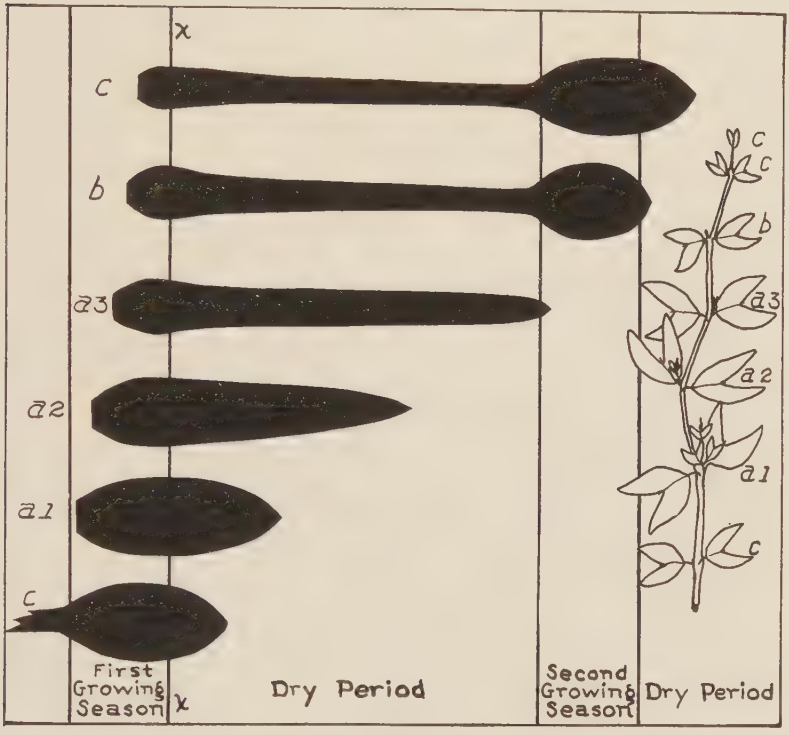

FIG. 6.—Schematic diagram representing life span and percentage water content of *Larrea* leaves. Relative water content indicated by thickness of bands: where the bands become narrower the water content is decreasing, and vice versa. The twig shows the relative positions of different leaf types at end of a growing season (at time indicated by x on left side of figure).

throughout their brief lives and are not drought resistant. The drought resistant *c* leaves look exactly the same except for their small size.[2]

[2] Figure 6 simplifies the true picture of percentage water content changes in the life of a leaf. Diurnal variations are not shown; the summer dry seasons are often broken by

It is to the *b* and *c* leaves that *Larrea* owes its reputation of being the most extreme xerophyte of the southern deserts of North America. The *c* leaves during a severe drought have a water content which is lower (except for air-dry plants or plant parts) than any other found reported in the literature, less than 50 per cent.[3]

Any *a* leaves (*a*2, *a*3) which are retained during a portion of the drought have also a very low water content, not very much more than the *c* leaves adjacent to them at the stem apex. Tremendously different in physiological capacity, these two leaf types are nevertheless quite similar in appearance, structure, and even in water content. In this respect *Larrea* differs from one of its companion species, *Encelia farinosa* (**26**).

A study of the degree of vacuolization and the chemical characteristics of the protoplasm of the *c* leaves as compared with that of the similar appearing but non-drought resisting *a* leaves might lead to a better understanding of the difference between these contrasting leaf types. Early development during a time of water shortage seems to engender drought enduring capacity, while attainment of maximum size and water content entails the loss of this capacity.

The degree of dormancy of the *c* leaves during the drought is of interest. With a water content about one-half their dry weight, metabolic activity must be definitely greater than that of air-dry leaves or seeds whose water content may be no more than one-tenth their dry weight. The appropriateness of the term "anabiosis" as applied to the condition of *Larrea* leaves (**18**) is thus questionable. The data on diurnal variation demonstrate that even in this state of minimum water content, *Larrea* leaves exhibit some diurnal varia-

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short periods of rain sufficient to result in water content increases, but not in growth. The time of occurrence and lengths of the rainy and dry seasons are different each summer. The general situation is as shown, however. June and July are usually the driest times in summer, and after early August rains another season of high temperatures and very low humidity is likely to occur. Even locally, however, there are apt to be conspicuous differences in the time and amount of precipitation.

[3] Water contents lower than half the dry weight are reported by THODAY (**37**) for some microphyllous South African ericads (*Passerina* spp.) but these data are for whole shoots, leaves, and stems together. Twigs of *Larrea* always have a lower water content than the leaves (fig. 4); and the older the stem, the lower the water content (unpublished data).

tion of the wc:dw ratio. No matter how much of this variation is due to dry weight change, fluctuation of the ratio implies activity. Experiments with moist chambers in the field (*q. v.*) indicate that the transpiration rate must be low corresponding to a very slow water intake. The significant fact, however, is not that water content and the rate of transpiration are low, but that some activity, some transpiration does occur; the leaves do not become air-dry.

Nodal differences may be due to age, position, and history. In some cases, as when comparing *a*1 and *a*2 leaves, the difference in age from node to node is slight, a matter of a few days only. In other cases newly developed leaves (*a*) are contrasted with leaves of a former growth period (*c*) which may therefore be months older. In any case, the difference in leaf wc:dw ratio from node to node is of much smaller magnitude than in the same leaf from season to season. In other words, changes due to external conditions are greater than those due to internal development. That the water content increases with advancing age indicates that the amount of water held by a leaf increases faster than does the weight of dry substance. When the cells have attained maximum size, further increase in the ratio must be due principally to dry weight loss. According to WALTER (40), the osmotic concentration also increases with age. This leads to the interesting deduction that the dry weight loss must be of the organic constituents, and more than enough to balance the increase in osmotic substances. A similar gradient in the percentage water content from younger to older leaves has been reported (41) for various plants, especially herbs. The second maximum (which according to YAPP and MASON occurs in buds, the minimum moisture content being in partially developed leaves) has been found in *Larrea* in only one test.

The reversed gradient of water content (decreasing with age) found during the four days of August 9–12, 1931, was exceedingly perplexing. The inconsistency with former results stimulated great care and effort to establish the true nature of the situation. A clue is afforded by some experiments, to be described later, with shoots in moist chambers. It was found that the youngest leaves have much the greatest water holding capacity, owing probably to their greater wall plasticity. During August 9–12 it may be supposed

that the plants had more than ample water to provide for transpiration. Their water content may thus have been conditioned principally by the extensibility of their cell walls. A descending curve of water content percentage from young to older leaves, even with constantly moist conditions, is reported to be the normal situation in many genera (**27, 17, 22**), but in *Larrea* is probably an unusual occurrence, being evidence of a superabundant water supply.

The characteristics of the diurnal changes are very evidently related to water conditions. The early day decrease and nocturnal increase are undoubtedly related to the changing rate of transpiration. On rare cloudy or rainy days the ratio does not sink so low. The diurnal minimum occurs late in the day more frequently in bushes of high water content, and earlier in the day in dry bushes. The interpretation of this situation may be that in drier bushes increasing water deficit *earlier in the day* reaches a point at which the colloids of the leaf tissues are in approximate equilibrium with their double environment, the taut tracheal sap and the desert atmosphere. Only a certain proportion of the total water content is seemingly subject to evaporation; this proportion is a larger quantity in bushes of higher water content; hence the turning point in the diurnal curve is reached later in the day in these bushes. Further, the magnitude of the diurnal change is related to the level of the water content. When the latter is only about 50 per cent of the dry weight and there has been no rain for months, the diurnal fluctuations are relatively slight, about 10 per cent of the early morning value; whereas when the water content is somewhat greater than the dry weight, the proportionate changes in the ratio may be more than 30 per cent, although more commonly about 20 per cent. Leaves of still higher water content show less proportionate diurnal change than leaves of medium water content; in the former, the water supply is evidently more nearly adequate to maintain an even water balance.

Probable changes of dry weight make the data relative to diurnal variations of the wc:dw ratio of only qualitative significance as regards water content. Diurnal changes of dry weight in semidormant drought-exposed leaves are probably less than for leaves of well watered plants in the relatively cool and humid environment of Cincinnati, where a small change in the dry weight of *Larrea* was demon-

strated. From three lines of evidence, (1) the Cincinnati experiments, (2) the correlation of water conditions with changes in the ratio, and (3) the lack of diurnal change of starch content in leaves of low ratio, the assumption seems justified that in the desert, especially in drier seasons, changes of the ratio are due much more to water content changes than to dry weight changes. During seasons of rain, however, when there is sufficient soil water to permit growth, dry weight as well as moisture undoubtedly undergoes diurnal fluctuations in direction opposite to that of moisture fluctuations, so that the ratio changes more than does the actual water content.

Diurnal variation of foliar moisture in *Larrea* is of about the same magnitude as for many other plants which come under WALTER's classification (40) of *euryhydric* species. These are unstable in percentage water content and sap concentration. Here belong such herbs as sunflower and potato (19), steppe plants such as *Plantago media* (11), and many desert plants (39, 25, 31, 19, 15). Contrasted with the euryhydric plants are those which are called by WALTER *stenohydric*, that is, having a very stable water balance. In the desert as in many other habitats euryhydric and stenohydric plants grow side by side.

A striking discrepancy appears (fig. 4) between the osmotic and the ratio values for the two experimental bushes Am and Bm. The level of the wc:dw ratio and the magnitude of its variation are greater for Am than for Bm; but the osmotic concentration of the sap is essentially the same in the two bushes, and follows conspicuously the same course. The difference between the two with respect to leaf size, vigor, and general appearance is paralleled by a difference in the wc:dw ratio, but not obviously in the osmotic concentration of the sap. These results emphasize the inadequacy of osmotic measurements alone in a study of water relations.[4]

It is impossible to assign definitely the reason for this discrepancy between osmotic and ratio values without supplementary data. The

[4] MALLERY (16) points out that the osmotic maximum occurs later in the day in bush Am (3 P.M.) than in Bm (1 P.M.), and that the variation in osmotic value is greater in Am. But these differences are certainly less striking than the close similarity of the osmotic curves for the two bushes, whereas the curves for the wc:dw ratio are not only of obviously different amplitude for the two bushes, but are on two distinct levels.

difference between the two bushes may be in water content, dry weight, or in both of these. Both chemical and anatomical investigations are required to give an adequate knowledge of water relations.

PISEK and CARTELLIERI (22) and OPPENHEIMER (21) have amplified their osmotic data with determinations of the percentage water content, and find in general a good correlation in the changes of the two quantities. In both these investigations, however, occasional discrepancies appear. For instance OPPENHEIMER reports that the stenohydric *Olea* undergoes more water loss than is indicated by the small rise in osmotic concentration; *Ceratonia* likewise may exhibit wilting phenomena but no concurrent increase in sap concentration. Chemical analyses of sap (30, 9) have shown that seasonal variations of osmotic value are due in some species (as *Ilex aquifolium*, *Hedera helix*, *Pinus sylvestris*) to sugar, in other species to both sugar and water (*Taxus baccata*), while in still others the variations may be due solely to water (*Buxus sempervirens*).

II. THE WC:DW RATIO UNDER EXPERIMENTAL CONDITIONS

THE WC:DW RATIO AT APPROXIMATE SATURATION.—To provide additional basis for comparison of naturally existing water content ratios, determinations were made of the values attained when shoots were inclosed in moist chambers. Some idea of the relation of the existing water content to that attained when the cells are distended to approximately their maximum size is afforded by use of the following method. Two branches are chosen, the moisture content of whose leaves by previous test has been found to be essentially the same. The leaves of one branch are picked for determination of the existing wc:dw ratio. The other branch is cut from the bush while the stem is held under water. The cut end of the severed shoot is kept under water while the whole is inclosed in a saturated atmosphere at a constant temperature for one or 24 hours, when the wc:dw ratio is determined. The data are not all strictly comparable because at times in the field it is necessary to deviate from the procedure just outlined. Two sources of error are thereby encountered:

1. The leaves picked for measurement of the original wc:dw ratio may not be strictly comparable with the leaves brought to saturation. A difference in the proportion of young to older leaves is espe-

cially to be avoided, because young leaves have a much higher water holding capacity.

2. The water content after 24 hours probably does not approach saturation to the same degree in all cases. The moist chambers used provide only an approximately saturated atmosphere. Some temperature changes are often unavoidable. Condensed moisture is sometimes visible as a film on glass or a mirror placed in the chamber. Moisture is never clearly discernible on the leaves, but for evaporation of any film that may have been present a period of exposure to dry air was often provided. An error in judgment as to the proper length of time for this would make some difference in the 24 hour ratio. The increases in the ratio are so great, however, that the errors involved are relatively insignificant.

Data relative to the saturation percentage water content, the amount of increase necessary to attain the 24 hour water content, and the rate of increase are presented in table IV and in figures 7 and 8.

Saturation water contents are found to be high above the naturally existing water contents, the latter being from less than one-third to only about three-fourths of the saturation values. The 24 hour ratio varies from bush to bush, with time of day, and with age of the leaf. Maximum values for the saturation ratio were obtained for bushes (Tb_5 and Cinti, table IV) which had a predominance of young, actively enlarging leaves. While in general higher original ratios increase to higher saturation values, often it happens that of two bushes the one with the lower water content is closer to saturation. The existing ratio is no indicator of the saturation ratio.

The 24 hour *increase* is greater in leaves of low water content. Expressed in terms of the original ratio, the increase is very much greater in drier bushes (fig. 7). Thus, according to the data in table IV, wc:dw ratios 0.40–0.70 increase 2-300 per cent, while ratios originally over 1.27 increase less than 100 per cent in 24 hours. In all cases, however, a very considerable increase has been found. The increases represent from 23 to 77 per cent of the 24 hour ratio.

The *rate* with which water enters the stems of cut shoots is of special interest. Figure 8 shows that after the first few hours in the moist chamber the saturation value has already been quite closely

approached, and that thereafter the ratio increases very slowly to the 24 hour value. After 24 hours the ratio continues the very gradual increase, although at 48 hours in some cases a lower value than at 24 hours has been found. Derangement of the normal metabolism of

TABLE IV

CHANGE IN THE LEAF WC:DW RATIO OF CUT SHOOTS IN A MOIST CHAMBER

BUSH	DATE (1931)	WC:DW RATIO			24 HR. INCREASE IN % OF ORIGINAL W.C.	ORIGINAL W.C. IN % OF 24 HR. W.C.
		ORIGINAL	AFTER 1 HR.	AFTER 24 HR.		
Cj	July 23	0.47	.....	1.76	274	26.7
Cj	July 15	0.58	.....	1.73	196	33.3
Cj	July 18	0.59	1.08	1.72	193	34.1
mn	June 19	0.48	1.42	2.08	334	23.0
Bj	July 9	0.57	1.27	2.03	257	28.0
Bj	July 9	0.61	1.32	2.12	248	28.7
Bj	July 9	0.55	1.27	2.03	270	27.0
Bj	July 11	0.62	1.30	2.15	246	28.9
Ej	July 21	0.57	.....	2.26	296	25.1
J	July 18	0.67	1.35	1.98	195	33.8
l	.....	0.68	1.44	1.97	190	34.4
U_{31}	June 27	0.73	1.45	1.93	164	37.8
b	June 18	0.97	1.82	2.18	125	44.8
m_2	July 4	1.01	1.49	2.18	116	46.4
m_1	July 4	1.02	1.46	2.07	103	49.4
e	July 4	1.27	1.91	2.22	75	57.2
e	July 4	1.33	1.69	2.23	68	59.6
C	July 10	1.33	1.73	2.30	65	57.8
E	Sept. 4	1.57	.....	2.76	76	56.9
E	Sept. 4	1.27	.....	2.50	97	50.8
E	Sept. 4	1.17	.....	2.26	93	51.7
E	Sept. 4	1.32	.....	2.38	83	55.5
Am	Aug 16	1.46	.....	2.52	73	57.9
Am	Aug. 17	1.78	.....	2.40	35	74.1
Am	Aug. 17	1.52	.....	2.54	67	60.0
Am	Aug. 17	1.77	.....	2.29	29	72.3
Am	Aug. 18	1.83	.....	2.48	36	73.8
Am	Aug. 18	1.59	.....	2.46	55	64.6
Tb_5	Aug. 12	1.78	.....	3.02	70	59.0
Cinti	July, 1933	1.94	.....	3.02	56	64.3

these cut shoots in a dark saturated atmosphere for periods longer than 24 hours makes doubtful the significance of 48 hour wc:dw values. The 1 hour percentage increase is roughly proportional to the 24 hour increase (fig. 7). Forty to 70 per cent of the total increase occurs during the first hour, and according to two tests (the

only ones made), 95 per cent of the 1 hour increase takes place within the first 15 minutes. Rapid increase of such magnitude in the wc:dw

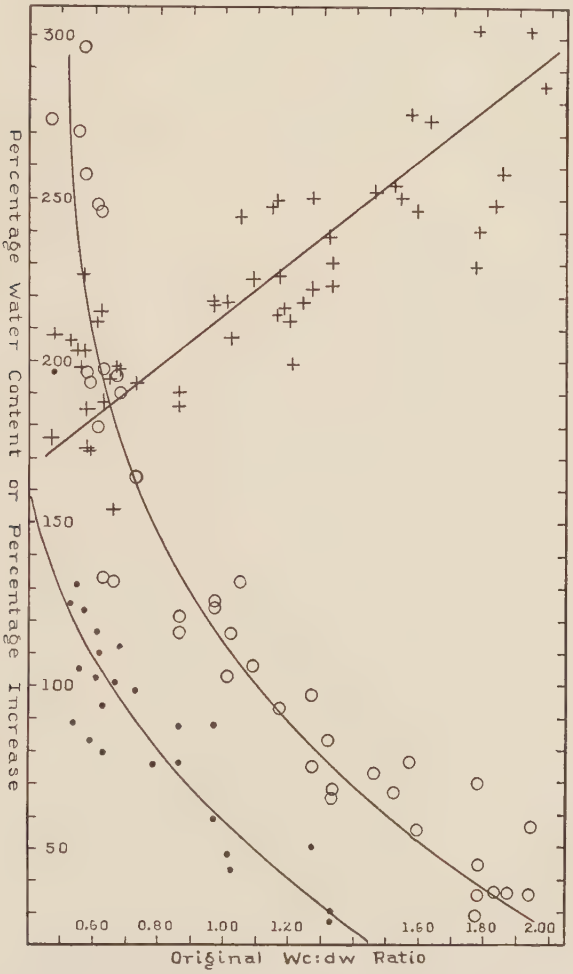

FIG. 7.—The 24 hour ("saturation") percentage water content (+) of leaves of bushes whose existing water contents ranged from 47 to 200%. For the same bushes the 24 hour *increase* (○) is shown as percentage of original value, and for some bushes the percentage increase after 1 hour (●). Curves drawn merely to indicate general regions in which the points fall.

ratio must be due principally to increase in water content; dry weight could not diminish so greatly in 15 minutes.

Further evidence in regard to how much dry weight may occur in leaves of severed shoots while in the moist chambers was obtained by the half-leaf method already described. The results of five experiments indicate that the change in water content is about eight times that of the dry weight.

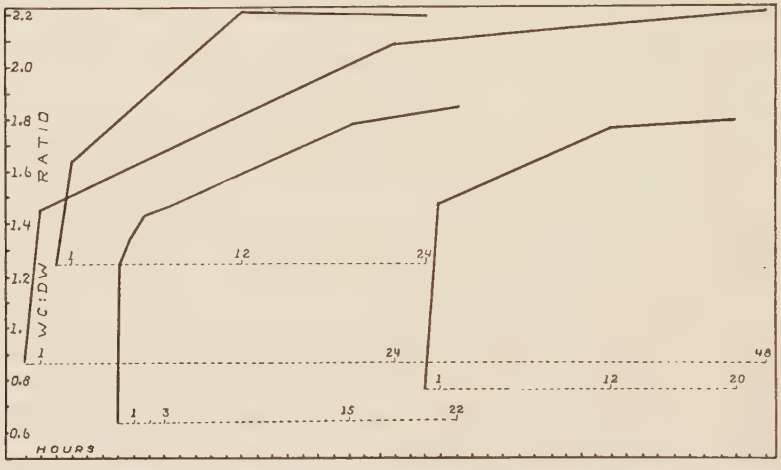

FIG. 8.—The wc:dw ratio of leaves of four shoots in a moist chamber at various time intervals after cutting from the plant and putting the stems in water.

Changes in leaves of the cut shoots during 24 hours in a moist chamber averaged 5.05% loss in dry weight

41.2 % increase in water (mg.)
46.1 % increase in wc:dw
14.5 % increase in water as percentage of fresh weight.

These experiments were conducted at the University of Cincinnati. In Arizona, higher temperatures may have led to greater change in dry weight, but probably not more than as much as to account for 15 per cent of the 24 hour increase in the ratio.[5]

EFFECT OF ELIMINATING TRANSPIRATION.—In another series of experiments with shoots inclosed by moist chambers, the stems were not cut and put in water but were left intact, the moist chambers

[5] A single test of the temperature effect in Tucson indicated no appreciable difference between 28° and 40° C. in the rate of increase of the wc:dw ratio.

being adjusted to the shoots in the field. Any increase in water content under these circumstances must be from the existing supply in the stems, roots, and soil. This type of experiment, carried out according to the procedure outlined below, proved to be a very simple and effective way of determining roughly the relative influences of transpiration and of insufficient water supply on the water balance.

A bell jar furnished with one or more wicks of paper or cloth toweling extending from the top down into a shallow pan of water is adjusted over the selected shoot. The pan of water rests on a support covered with one or more layers of waterproofed fabric which is fitted snugly around the stem of the shoot and up around the base of the bell jar, and made tight with elastic tubing. Two coats are fitted over the bell jar: one of thick paper felting for heat insulation, and an outside coating of glistening white paper to minimize sunlight absorption. The temperature within the chambers does not differ significantly from the external temperature. Dew formed in the chambers as a result of temperature fluctuation has always to be evaporated before leaves are picked for determination of wc:dw ratio. To outward appearance, shoots of *Larrea* which have been in a moist chamber even as long as 2 or 3 days appear normal in every respect. The leaves, however, doubtless suffer some loss of their substance owing to continued respiration in the absence of photosynthesis, so that the wc:dw ratio may be expected to increase even independently of any increase in water content.

In contrast to the experiments with cut shoots, no sudden large increase in water content takes place. Indeed, except in bushes k31, x, y, and z (table V), the increase in the wc:dw ratio as a result of the use of moist chambers in the field was small, and in many cases may have been due to dry weight decreases.

When only one shoot of bushes t31, h31, and bl was inclosed by a moist chamber, similar diurnal fluctuations were shown by both exposed and experimental leaves, although the latter were protected from transpiration. This implies that protected leaves lost water during the day to nearby exposed transpiring leaves. Transpiration increases the osmotic concentration of the sap of exposed leaves over that of protected leaves so that the former can draw on the protected leaves for part of their water supply. This translocation of water

from one shoot to another indicates a deficient soil water supply, and did not occur when there was ample, as for bushes g, bs, and k31 (table V). Bushes x, y, and z (fig. 9) show both situations. By com-

TABLE V

CHANGE IN LEAF WC:DW RATIO OF INTACT SHOOTS IN A MOIST CHAMBER

BUSH	DATE	NUMBER OF WC:DW DETERMINATIONS	VARIATION LIMITS OF WC:DW RATIO		24 HR. "SAT." WC:DW	CHARACTERISTICS OF THE WC:DW RATIO OF THE PROTECTED LEAVES, ETC.
			EXPOSED	PROTECTED		
Ej	July 15–28 1931	32	0.57–0.61	0.58–0.66	1.70	Whole bush covered July 21; increase over control, and variations slight
Aj	July 9–13 1931	10	0.60–0.62	0.65–0.66	1.87	Readings at 3 P.M.
t31	June 26–28 1931	20	0.74–0.83	0.75–0.86	.....	Follows control
g	June 14–15 1930	10	0.74–0.83	0.90–0.93	1.93	Does not follow control; readings at 5:30 A.M. and 2:30 P.M.
bs	July 27–30 & Aug. 3–4 1930	18	0.67–0.90	0.80–1.02	.....	Does not follow control; no midday drop
h31	July 2–11 1931	18	0.83–1.02	0.94–1.07	1.77	Follows control, when not entirely covered
k31	July 1–5 1931	15	0.92–1.01	0.98–1.37	.....	Does not follow control; marked increase when entirely covered
bl	June 17–18 1931	14	0.90–1.09	0.88–1.09	2.18	Follows control
x, y, & z	Aug. 26–Sept. 5 1931	66	0.99–1.50	1.01–2.16	2.45	Follows or not, depending on conditions (see fig. 9)

parison of the curves for x and y for August 26–27 with those of all three bushes for September 1–2, the effect of rains which fell prior to September 1 is indicated. It is interesting to compare also bushes g and t31 (table V), exposed shoots of which had the same leaf water content and diurnal fluctuation, 0.74–0.83. The water content of the protected leaves of bush h31 decreased at midday just as did the exposed control, while the wc:dw ratio of protected leaves of bush g

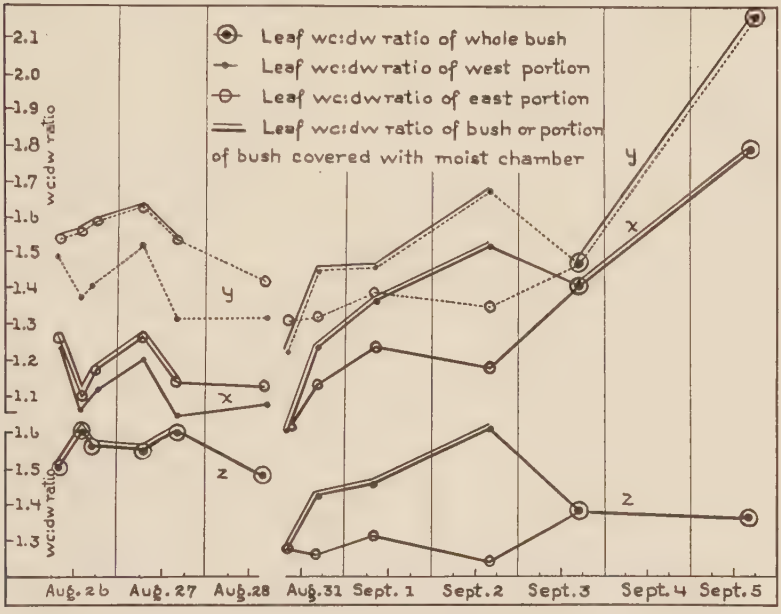

FIG. 9.—Variations in wc:dw ratio of leaves of bushes x, y, and z when partly and when completely protected from transpiration by the use of moist chambers in the field.

remained about constant or increased slightly. The wc:dw ratio of the exposed shoots alone fails to indicate the better water supply of bush g.

The effects of water translocation can be avoided by covering entire bushes. This was done for the small bushes Ej, h31, k31, x, y, and z. Even when completely covered, the resulting increase in the wc:dw ratio for bushes Ej, h31 (table V) and for bush z, Aug. 26–27 (fig. 9), was surprisingly small. The very slow reduction of the water tension, when transpiration is held at minimum by means of a moist

chamber, is convincing evidence of a deficient water supply. Transpiration, having once reduced the water content to a low level, is relatively much less important than the diminishing water supply in maintaining a low wc:dw ratio in these bushes. It is to be emphasized that this is the usual situation; not transpiration, but insufficient soil water is what maintains the low moisture content in *Larrea* leaves.

On the other hand, when there is enough water for growth, transpiration is very active in restricting the wc:dw ratio. Thus Sept. 3–5, when all of the shoots of bushes x and y (fig. 9) were covered, a comparatively rapid increase took place while bush z, entirely exposed, suffered a slight decrease. Here again the inadequacy of the wc:dw ratio of exposed leaves alone to indicate the situation is seen by comparing the data for h_{31} and k_{31} (table V). The wc:dw ratio of the leaves of both bushes is about the same. The moist chamber experiments, however, show that water is relatively more available to the leaves of bush k_{31} which when covered exhibited a markedly increased ratio.

General discussion.—The data presented in this section demonstrate that of two bushes having the same water content, one may be much further from its saturation water content or may have a significantly smaller water reserve than the other. The use of moist chambers in the field on intact plants may prove to be a valuable method of investigation of the water balance of other species.

The amount of deviation of the natural wc:dw ratio from the saturation value is related somewhat to the capacity of a plant to endure drying, but is clearly not to be taken as an indicator of this capacity. The saturation wc:dw ratio is never attained in nature; it is not a constant; and it is higher than the existing ratio partly because of dry weight changes (**39**). The inconstancy of the saturation water content is easily understood, for the total water capacity of a cell depends first upon the varying difference between its vapor tension and that of its environment, and second, upon the extensibility of its walls. The relatively high capacity shown by young leaves is no doubt due to the greater plasticity of their walls.

The great rapidity of water intake when shoots are cut under water (fig. 8) evidently indicates that a high tension normally exists

in the hydrostatic system of the shoots. The elimination of transpiration from intact shoots does not immediately relieve this high tension. The highest ratios attained by leaves on intact shoots, even though these are surrounded by a nearly saturated atmosphere, are still well below the saturation ratios (table V).

TABLE VI

DIFFERENCE BETWEEN EXISTING AND SATURATION WC:DW RATIO IN PERCENTAGE OF SATURATION VALUE

REFERENCE	REGION	HABITAT	PLANT	PERCENTAGE DIFFERENCE ("DEFICIT")
(11)	South Russia	Swamp	Alisma plantago	0.5–7.7
		Meadow	Rumex confertus	4–9
		Steppe	Plantago major	19–40
(26)	Arizona	Desert	Encelia farinosa	–50
(25)	Arizona	Encinal	Quercus emoryi	28–29
			Arctostaphyllos pungens	17–27
(37)	South Africa	Desert	Passerina spp.	–55
(31)	Egypt	Desert	Non-succulent plants	35–56
			Mesembryanthemum	19
(33)	Hungary	Alkali steppe	Achillea millefolium	4–19
			Epilobium montanum	11–16
		Baltic sea-coast	Calluna vulgaris	–23
(34)	Swedish Lapland	Arctic tree limit	Shrubs and herbs	4–10
(22)	Mid-Europe	Alpine tree limit	Ericaceae spp.	"0"–30
		Shade	Mercurialis perennis	4–12
		Sun	Coronilla varia	1–33
(21)	Jerusalem	University campus	Ficus elastica	–16
			Rosmarinus officinalis	30–65
RUNYON	Arizona	Desert	Larrea tridentata	23–77

In table VI are given approximate values for the percentage difference between the existing and saturation wc:dw ratios for various plants. The methods used by the investigators have differed, so that the data are only roughly comparable. The terms water deficit and saturation deficit have often been used to designate this difference.

It is seen that *Larrea* may endure a water content further from its maximum water holding capacity than any other plant reported.

Summary

1. From various lines of evidence it is shown that the dry weight of *Larrea* leaves undergoes diurnal fluctuations when there is enough water for growth; but under the usual desert conditions the dry weight probably changes very slowly, water conditions being chiefly responsible for the fluctuations of the wc:dw ratio. However, the usual disregard of dry weight changes in literature pertaining to percentage water content, and the use of the fresh weight basis for expressing the data, have often led to erroneous interpretations.

2. The amount of moisture relative to dry weight in leaves of *Larrea* differs with age, position on the stem, season, habitat, and time of day.

3. Apart from seasonal influences, water content increases with the age of the leaves, except during rare periods of ample soil water, when the younger more plastic leaves have the higher water content.

4. During periods of growth the water content of the foliage is not lower than that of many mesophytic tree leaves: between one and two times the dry weight. During severe drought the leaves may endure a water content less than half the dry weight, a value lower than that reported for any other seed plant (except air-dry plants or plant parts).

5. Leaves at adjacent nodes, although practically indistinguishable in appearance and structure, may differ greatly in physiological capacity, depending upon the water conditions existing during their development. Three leaf types, *a*, *b*, and *c*, may be recognized. The *a* type, most abundant, has a comparatively high water content, and very little or no drought resistance. The *b* type is intermediate in all respects to *a* and *c*. The *c* leaves are resistant to drought, have a lower water content during their expansion from the bud, and a longer life span than the *a* leaves.

6. Development during a time of water shortage seems to engender drought enduring capacity, while attainment of maximum size and water content entails loss of this capacity.

7. The drought enduring *c* leaves have two periods of enlargement,

separated by a season of drought-induced dormancy. This dormancy is not complete, as is shown by the facts that the leaves are not air-dry and that a slight diurnal variation of the wc:dw ratio occurs.

8. In general, osmotic changes follow in a reciprocal manner the diurnal water content changes, but the latter may disclose differences not evident in the osmotic measurements alone.

9. Large and very rapid increases in the water content of the leaf occur when the stems are cut and allowed to remain under water, the leaves being inclosed by a moist chamber. This implies a high tension in the tracheal sap.

10. The saturation water content is higher above the naturally existing water content for *Larrea* leaves (*b* and *c* types) than for any other species for which data have been found in the literature.

11. By following the changes of the wc:dw ratio of leaves of bushes partly or completely covered with moist chambers, the adequacy of the water supply to the leaves may be judged.

12. Under usual conditions a deficient water supply rather than transpiration is the crucial factor limiting the moisture content; while after rains the rate of transpiration may be very high and the chief cause of the considerable difference between the saturation and existing water contents.

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